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FINAL REPORT

GRANT #: N00014-99-10296

PRINCIPAL INVESTIGATOR: Dr. Diane McKnight

INSTITUTION: Institute of Arctic and Alpine Research,  
University of Colorado

GRANT TITLE: Fulvic acid oxidation state detection using  
fluorescence spectroscopy

OBJECTIVE: To investigate and understand the processes by which microorganisms in marine and coastal sediments can use quinone moieties in humic substances as electron acceptors in the degradation of recalcitrant organic material under anoxic conditions.

APPROACH: All humic substances have fluorescent properties and fluorescence spectroscopy can indicate differences in precursor organic source of humic substances. Our approach is to use fluorescence spectroscopy to elucidate the oxidation state of quinone moieties in humic substances at natural concentrations found in sediment interstitial waters. Fluorescence can be readily detected at fulvic acid concentrations (2-5 mg C/L) found in most natural waters. We hypothesized that humic substances in the oxidized and reduced states would have different fluorescence spectra. We focused on fulvic acids isolated from three sites in San Diego Bay with differing sources of sedimentary organic carbon. We isolated fulvic acids from sediments at these sites and studied their fluorescence characteristics before and after microbial reduction.

ACCOMPLISHMENTS: Characterization data was obtained for the San Diego Bay fulvic acids in order to compare them to fulvic and humic acids from other sources. These data were obtained on San Diego Bay sediment fulvic acids that were isolated after being stored at 4°C for 4 months (referred to as "fresh" fulvic acids). The fluorescence indexes, electron accepting capacity and percent aromaticity of fulvic acids varied among the three sites indicating differences in sources of organic material. The electron accepting capacity of the isolated San Diego Bay fulvic acids also varied greatly with source. Fulvic acid from Paleta Creek sediment had an electron accepting capacity close to the high value of the organic rich Nymph Lake sediment fulvic acid. In contrast, fulvic acid from Coronado Cayes and Shelter Island sediment had a very small electron accepting capacity. The electron accepting capacity of these fulvic acids was inversely related to their fluorescence indexes.

For all three San Diego Bay sediment fulvic acids, the Excitation Emission Matrices (EEMs) of the reduced fulvic acids was considerably different from the EEMs of the fresh fulvic acids and were similar to that of reduced AQDS (AHDS). The longer excitation-emission maximum almost completely disappeared in the EEM of the Coronado Cayes reduced fulvic acid and the shorter excitation-emission maximum significantly red shifted. In the EEMs of the reduced samples for both the Paleta Creek and Shelter Island, the breadth of the longer excitation-emission maximum is significantly diminished and is shifted to a shorter excitation wavelength. The shorter excitation-emission maximum of the reduced Shelter Island sample is significantly broader and red-shifted as compared to the fresh sample; however, no change is observed in the shorter excitation-emission maximum of the Paleta Creek reduced and fresh samples. In all cases, the intensity of the reduced fulvic acids was less than the intensity of the fresh fulvic acids. In addition, the EEMs of the control experiments were similar to that of the fresh fulvic acids. From these results, we inferred that for the Coronado Cayes samples more of the fluorescence was due to functional groups involved in electron transfer by microbes than in the Paleta Creek and Shelter Island samples. In combination with the similarity of the EEMs of AQDS and AHDS to the EEMs of the fresh and reduced sediment fulvic acids, the dramatically different EEMs of the fresh and reduced fulvic acids suggest that quinone groups are partially responsible for the fluorescence found in humics.

In order to obtain a larger quantity of material in hopes of studying this process in greater detail, fulvic acids were extracted from San Diego Bay sediment (Paleta Creek and Coronado Cayes sites) that had been stored at 4°C for 13 months (referred to as "aged" fulvic acids). The EEMs of the aged fulvic acids were dramatically different than the EEMs of the fresh fulvic acids, and more resembled the EEMs of the reduced fulvic acids (third column of Figure 3). Microbial activity would have continued at this temperature. In addition, the supply of available Fe(III) could have become depleted in the sediment, leaving the fulvic acids to act as the terminal electron acceptor. Interestingly, addition of Fe(NO<sub>3</sub>)<sub>3</sub> to these aged fulvic acids results in a blue-shift of their corresponding emission peaks back to near their original location before reduction.

**CONCLUSIONS:** Field studies of electron shuttling processes involving microorganisms in sediments and humic substances have been constrained by the lack of methods to characterize the oxidation state of quinone moieties in humic substances at natural concentrations. All humic substances have fluorescent properties and fluorescence spectroscopy can indicate differences in precursor organic source of humic substances. We have shown that the quinone moieties

responsible for electron transfer reactions contribute significantly to the fluorescence of humic substances. Further we use fluorescence spectroscopy to elucidate the oxidation state of quinone moieties in humic substances at natural concentrations found in sediment interstitial waters in San Diego Bay.

SIGNIFICANCE: Recent research on microbial electron transfer involving humics has revealed the ecological significance of electron transfer within humics. Now through the use of fluorescence spectroscopy to measure another universal characteristic of humics, we can now investigate the biogeochemistry of electron transfer reactions in marine and freshwater environments. The development of this fluorescence method for evaluating the redox state of humic substances in anoxic marine sediments will allow for the assessment of the potential for organic and trace metal contaminants to be microbially transformed over time in the sediment environment.

AWARD INFORMATION: Promoted to full professor at the University of Colorado in the department of Civil, Environmental and Architectural Engineering.

PUBLICATIONS AND ABSTRACTS:

1. Klapper, L., D.M. McKnight, J.R. Fulton, E.L. Blunt-Harris, K.P. Nevin, D.R. Lovley, P.G. Hatcher. (2002) Fulvic acid oxidation state detection using fluorescence spectroscopy. Environm. Sci. Technol. 36:3170-3175.
2. McKnight, D. M., D.T. Scott, D.C. Hrncir, D.R. Lovley. 2001. Photochemical and microbial processes influencing iron-humic interactions in stream and lake sediments. In: Humic Substances and Chemical Contaminants, pg 351-369. Soil Science Society of America.
3. McKnight, D. M., Hood, H, and L. Klapper. (in press). Trace organic moieties of dissolved organic material in natural water. In (Findlay and Sinsabaugh, eds.) Aquatic ecosystems: Interactivity of Dissolved Organic Matter.
4. McKnight, D. M. 2000. Use of fluorescence for characterizing source and speciation of aquatic humic substances, presented at American Chemical Society, Division of environmental chemistry, summer meeting, August 2000.
5. McKnight, D. M. 2000. Variation in biogeochemical interactions of iron and fulvic acid in sediments of San Diego Bay, presented at American Geophysical Union, Fall 2000 meeting.
6. McKnight, D. M. 2000. Trace organic moieties of dissolved organic material in natural waters, invited talk presented at Institute of Ecosystem Studies Workshop on Dissolved Organic Material in Surface Waters, May 2000.

7. McKnight, D. M. 2000. Interactions between humic substances and iron in aquatic ecosystems, keynote lecture presented at Humic Substances Seminar IV, Boston, MA, March 2000.

8. McKnight, D. M. 2000. Organic matter sorption on oxide surfaces; from colloids to catchments, keynote talk for session on mineral-organic interactions in aqueous systems presented at Goldschmidt Conference, Hot Springs Virginia, May 2001.

9. McKnight, D. M. 2001. Humic substances as electron acceptors: an important feedback in aquatic ecosystems, invited talk presented at University of Illinois at Urbana-Champaign, Department of Geology, September 2001.

10. McKnight, D. M. 2001. Humic substances in aquatic ecosystems: detection of source and redox state by fluorescence spectroscopy, invited talk presented at Observatoire Oceanologique, Villefranche-Sur-Mer, March 2001.

11. McKnight, D. M. 2002. Humic substances and electron transport reactions in aquatic ecosystems: the role of quinone moieties. Plenary talk at Natural Organic Matter Workshop, University of Minnesota, March 2002.